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Project Title: In-situ Containment and Treatment: Engineering Cap Integrity and Reactivity
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A. Project Goals

Alternative materials designed to sequester or transform sediment contaminants have recently been incorporated into *in situ* caps to create "reactive caps". The purpose of reactive caps is to remove contaminants from the aqueous phase during transport through the cap, theoretically eliminating break through into the overlying water. Thus, unlike a traditional sand cap, they can be applied at sites subject to advective flow. To date, the development of reactive caps has focused on physicochemical methods of contaminant removal such as abiotic reactions or sorption. One limitation of such physicochemical reactive caps is that they are subject to fouling (abiotic and biological), which can reduce reactivity with time. A second limitation of physicochemical reactive caps is that they inherently possess a finite capacity for reaction/sorption, creating the need to replenishment of the reactive/sequestering material over time to maintain reactivity. The development of a reactive cap that can continuously treat mobile contaminants would eliminate the risk of contaminant break through due to loss of reactivity, decrease the possibility of contaminant resuspension during maintenance operations, and potentially lower treatment costs. The emplacement of microorganisms capable of contaminant biotransformation within a cap provides an opportunity to meet this need. The overarching goal of this research is to develop a laboratory-scale biologically-based reactive (i.e., bioreactive) *in situ* cap.

Prior to the development of a bioreactive cap, fundamental research is needed on the natural bioattenuation of chlorinated solvents in capped sediments and what supplements are needed to maintain a microbial community in the cap. Research discussed herein is intended to provide information on the general topics: (1) the intrinsic biotransformation of chlorinated solvents within capped sediment subject to advective flow (i.e., what contaminants would enter a bioreactive cap); and (2) the limitations on microbial reactivity toward contaminants within a cap (i.e., what ingredients are needed to maintain a successful bioreactive cap). The first experimental objective of the research is to determine if Anacostia River sediment can intrinsically dechlorinate PCE to nontoxic end products. This objective needed to be met in order to gauge the potential for natural bioattenuation process occurring within the sediment under environmentally relevant conditions and dictated how future experiments were designed. To address this objective, microcosms and sediment columns were be prepared using wet Anacostia River sediment, with care being taken to accurately simulate natural conditions. Molecular techniques were used to characterize the dechlorinating community and to probe for other microbial species of interest.

After identifying which contaminants will enter a cap, research will be performed on how to maintain biological activity within the cap. The potential for a biologically-based cap to maintain reactivity over time provides an advantage over physicochemical-based reactive caps. Sediment porewater entering the cap, however, may need to be supplemented with organic substrates and nutrients in order to stimulate and maintain the microbial community in the cap. Such techniques have proven successful in groundwater aquifer bioremediation. Experiments were conducted using sediment effluent as the mineral media, carbon source, and energy source

while screening a suite of amendments (e.g., vitamins, electron donor) to determine if/what limitations on microbial activity are present. A bioreactive *in situ* cap was simulated by supplying sediment effluent to a sand column inoculated with a microbial culture.

B. Project Rationale

Due to the cost of dredging, and concerns in the management of dredge spoils, it is imperative that *in situ* solutions be developed for the management and treatment of contaminated sediments. Capping, the placement of a clean layer of sediment over the contaminated bed, is an attractive solution in many cases, as it is effective in rapidly isolating contaminants from benthic organisms and can improve sediment stability. However, there are concerns regarding the long-term effectiveness of caps when placed at regions subject to contaminated groundwater seeps. Work has been performed to better understand and predict the effectiveness of caps during groundwater seep conditions. Our studies focus on the development of sediment caps where biologically-mediated contaminant detoxification is achieved during the transport through the cap. The ultimate outcome of the research is to incorporate sustainable reactive processes in a reactive cap.

C. Summary of Results

Microcosm experiments demonstrated that microorganisms indigenous to Anacostia River sediment were capable of completely dechlorinating PCE to the nontoxic end products of ethene and ethane under methanogenic conditions. The addition of exogenous reducing equivalents, media, vitamins, and carbon sources to the microcosms was not required for complete dechlorination of PCE. Increasing the amount of reducing equivalents supplied to the microcosms resulted in decreased times required for complete dechlorination, suggesting possible electron donor limitations within the system. Analysis of the microbial community revealed several dechlorinators, including multiple *Dehalococcoides* strains. The overall conclusion from the microcosm experiments was that it is possible for complete PCE dechlorination to occur within Anacostia sediment, but a lack of readily available electron donor may limit the extent of dechlorination. A 1-D upflow sediment column simulating capped sediment confirmed these results, as complete dechlorination of dissolved-phase PCE was only achieved when electron donor (lactate) was added to the influent. Prior to amending with lactate, a mixture of PCE, TCE and *cis*-dichloroethene (DCE) was detected in the column effluent along with very low concentrations of methane and hydrogen. The addition of lactate stimulated the dechlorinating community, leading to complete dechlorination and biologically enhanced desorption of chloroethenes from the solid phase. Methanogenesis, hydrogen production, and iron reduction were also stimulated by the addition of lactate. Complete dechlorination ceased when lactate addition was stopped, although ethene was still observed in the effluent. The column study demonstrated that the capacity of Anacostia River sediment to serve as a natural biobarrier for the detoxification of PCE groundwater plumes may be limited by a lack of electron donor, and that the natural attenuation of chloroethenes may be impaired due to the presence of an *in situ* cap.

Research has also been completed focusing on understanding what metabolic amendments are required to sustain a dechlorinating culture fed contaminated sediment effluent. Two sand columns were connected in series to a sediment column performing only partial dechlorination of PCE. The sediment effluent, composed of *cis*-DCE, VC, and ethene, served as the influent for the sand columns. The sand columns were inoculated with a PCE to ethene dechlorinating mixed culture prior to connection with the sediment column to simulate the

emplacement of a bioreactive sand cap. When influent was consisted solely of sediment effluent, the dechlorinating culture within the first sand column was not able to maintain activity. However, when electron donor (lactate; 5 mM) was supplied to the sediment effluent, complete dechlorination to ethene was observed at relatively fast residence times (0.5 days) within the second bioreactive sand column. The results of the completed research suggest that electron donor availability and influent chloroethene flux can limit the success of a dechlorinating sand cap.

D. Detailed Discussion of Research

I. Materials and Methods

Chemicals. PCE (99+%, Sigma-Aldrich, St. Louis, MO), TCE (99.5%, Sigma-Aldrich), *cis*-DCE (97%, Acros Organics, Morris Plains, NJ), *trans*-DCE (99.7%, Acros Organics), and 1,1-DCE (99.9%, Acros Organics) were obtained in neat liquid form. Vinyl chloride (8%/ N_2 balance), ethene (99.5%), ethane (99.5%), and methane (99%) were obtained from Matheson Tri-gas (Parsippany, NJ). Sodium bicarbonate, potassium chloride, magnesium chloride, calcium chloride, sodium lactate syrup (60% vol/vol), ferric chloride, ferrozine (98+%), and potassium bromide were all purchased from Fisher Scientific. Hydrogen gas (80%/20% CO_2) was purchased from Airgas (Atlanta, GA) and glacial acetic acid (ACS grade) was obtained from Acros Organics.

Sediment. Surficial sediment from the Anacostia River, Washington, D.C., was used as the sediment for all experiments. Sediment from the river has previously been characterized and found to contain elevated levels of PCBs, PAHs, metals, and organic pesticides (*1*). The wet sediment was homogenized in the laboratory by mechanical mixing with large debris and shells removed, transferred to quart-sized mason jars, and stored in the dark at 4° C. Physicochemical characterization of the homogenized sediment included tests to determine wet bulk density, particle density, gravimetric water content, porewater salinity, and particle size distribution. Total organic carbon (TOC) in dried sediment samples was obtained with a Shimadzu TOC-5050A Total Carbon Analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD) equipped with non-dispersive infrared detector with catalytic combustion at 680° C.

PCE Sorption. The sorption of PCE to Anacostia sediment was observed in a microcosm constructed within a 70 mL serum bottle loaded with wet sediment, simulated groundwater, and neat PCE. The sediment was sterilized by autoclaving for one hour on two consecutive days prior to PCE addition. Partitioning of PCE into the gas phase was predicted from the volume of microcosm headspace and Henry's coefficient for PCE, 0.723 (*2*). Partitioning onto the solid phase was modeled using the mass of solid sediment material and the linear distribution coefficient (K_D) for PCE, which was calculated to be 13.25 mL·g^{-1} from the product of the measured organic carbon content of the sediment (5.22%; Table 1) and an average literature K_{oc} for PCE (265 mL·g^{-1} (*3*)). Nonequilibrium sorption was described using a one-site chemical nonequilibrium model:

$$C_{w,t} = C_{w,eq} - (C_{w,eq} - C_{w,0}) \exp^{(-kt)}$$

where $C_{w,t}$ is the aqueous phase concentration (mg·L^{-1})at time t (hr), $C_{w,eq}$ is the calculated equilibrium concentration (mg·L^{-1}), $C_{w,0}$ is the initial aqueous phase concentration (mg·L^{-1}), and k is the sorption rate coefficient (hr^{-1}) (*4, 5*). The value of k was fitted to the data to minimize the

root mean square error at each data point and incorporates both the dissolution of neat PCE into the aqueous phase, as well as the sorption of aqueous PCE onto the sediment. The model was used to determine solid phase PCE concentrations during mass balance calculations of the biologically active microcosms.

Microcosms

PCE Microcosms. Microcosms were constructed in duplicate by loading serum bottles (70 mL) with wet river sediment and simulated groundwater under anaerobic conditions (5% CO_2/ 5% H_2/ 90%N_2) in a glove-box (Coy Laboratory Products, Grass Lake, MI). The ratio of wet sediment to groundwater in the microcosms was 1:1.5 (v/v) following the method of Lorah and Voytek (*6*). The composition of the simulated groundwater was slightly modified from that described by Dries et al. (*7*), and consisted of 3.5 mM $NaHCO_3$, 0.1 mM KCl, 0.25 mM $MgCl_2$, and 0.75 mM $CaCl_2$. The microcosms were sealed with Teflon-lined butyl rubber stoppers and aluminum crimp caps. Neat PCE was introduced into the microcosms with a 10 µL syringe and the microcosm mass difference was recorded following injection to determine the mass of PCE loaded. The target aqueous phase PCE concentration was approximately 0.08 mM after partitioning among gas, solid, and aqueous phases.

Microcosms were supplied with dissolved acetate and hydrogen gas ranging from 0 to 2.5 mmoles reducing equivalents to test their effect on the rate and extent of dechlorination (Table 2). Acetate was added from an anaerobic, sterile stock solution of acetic acid in simulated groundwater, and hydrogen gas was added directly to the microcosm headspace. When dechlorination was complete (i.e., conversion to ethene/ethane), the microcosms were sparged with N_2, recapped under anaerobic conditions, and respiked with neat PCE. No mineral media, carbon sources (with the exception of the acetate microcosms), nor vitamins were added to the microcosms other than what was provided by the sediment in order to replicate environmental conditions. Duplicate active control microcosms were established without PCE to ensure that all observed ethenes were experimentally derived. Bioaugmented microcosms were also constructed with a 5% inoculum of a PCE to ethene mixed culture (*8*) to test the effect of excess dechlorinating cells on dechlorination rate. Sterilized microcosms were prepared as described above but autoclaved at 121° C for 1 hour on two consecutive days prior to PCE addition to ensure that any dechlorination observed in the active reactors was the result of microbial activity and not due to abiotic PCE transformations (*9-11*). A lumped rate of neat PCE dissolution into the aqueous phase, headspace partitioning, and sorption in the sterilized controls was obtained from a nonequilibrium sorption model (*4*) and used to estimate PCE mass on the solid phase during mass balance calculations (see discussion above under *PCE Sorption* heading). All microcosms were wrapped in foil and incubated at 20° C on an orbital shaker at 150 rpm. Chlorinated ethenes, ethene, ethane, and methane concentrations were determined from headspace samples of the microcosms.

DCE Screening Microcosms. A large PCE enrichment culture was established in a 500 mL media bottle modified with a glass side port capped with a mini-inert valve for headspace sampling. Stainless steel tubing extended through the screw-top cap of the bottle while remaining gas tight for the removal and replenishment of aqueous solution. Wet sediment and DCB-1 media were initially loaded into the media bottle under anaerobic conditions. The media has been described previously (*12*) and was supplemented with Wolin vitamin solution. Approximately 260 mL of aqueous volume (sediment porewater and media) and approximately 140 mL of solid volume were added to the reactor. Lactate was added from an anoxic, sterile stock to achieve an aqueous concentration of 5 mM. Aqueous PCE was introduced into the

culture through the side port from a saturated, anoxic, and sterile stock solution to achieve concentrations of approximately 0.03 mM. The reactor was wrapped in foil and maintained at 20° C with a stir bar continuously mixing the culture. Headspace samples from the side-arm allowed for monitoring of PCE dechlorination. Following each dechlorination cycle of PCE to ethene/ethane, 60 mL of aqueous solution was removed from the culture and discarded. The reactor was replenished with an equivalent volume of media containing vitamins, lactate, and aqueous PCE.

A large sediment-free culture was established in a similar reaction vessel by transferring 60 mL of aqueous solution from the large sediment culture into 340 mL DCB-1 media. Lactate (5mM) and Wolin vitamins were supplemented to the media. Aqueous PCE is again provided to achieve a concentration of nominally 0.03 mM, with dechlorination being monitored via headspace samples. Once dechlorination is complete, 100 mL of aqueous solution is removed from the culture, discarded, and replenished with media, vitamins, lactate, and aqueous PCE. This sediment-free culture is also stirred continuously by a stir bar, wrapped in foil, and maintained at 20° C.

The sediment-free enrichment culture was tested for its ability to dechlorinate alternative electron acceptors other than *cis*-DCE, specifically 1,1-DCE and *trans*-DCE. A 20 mL aliquot of sediment-free culture was removed from the large vessel following a completed dechlorination cycle and transferred into a pre-capped, anaerobic 70 mL serum bottle. The aliquot was sparged for 15 minutes to remove volatile components and the bottle headspace was sampled to quantify any residual chloroethenes. Lactate stock was added at 5 mM from an anaerobic, sterile stock of 100 mM of lactate in simulated groundwater. DCE (either 1,1-DCE or *trans*-DCE) was added from a methanol stock to achieve a concentration of approximately 0.45 mM. DCB-1 media and Wolin vitamins were added to obtain a final volume of 50 mL. Killed controls were prepared in similar fashion but were autoclaved 121° C for 30 minutes on two consecutive days prior to adding the respective DCE isomer. A positive control was also prepared in the same manner as the active DCE cultures but with PCE as the electron acceptor since this was how the culuture was normally maintained. All cultures were wrapped in foil, stored inverted at 20° C on an orbital shaker set at 150 rpm, and monitored via headspace sampling.

Aerobic VC Oxidation Microcosms. VC oxidizing microcosms were prepared with wet sediment and simulated groundwater in the same manner as described above for the PCE microcosms, but under an aerobic atmosphere and with VC provided as the chlorinated ethene. VC was introduced into capped microcosms with a gas-tight syringe, with monitoring via headspace sampling. When no VC was observed in the headspace, the septa and cap were removed and the microcosm was placed in a fume hood and allowed to remain open to the atmosphere for 30 minutes to replenish oxygen to the system. A septa and cap was then placed back on the serum bottle and VC was respiked into the microcosms.

DNA Extraction and PCR. Microbial characterization was performed on a microcosm fed PCE, acetate, and hydrogen which was dechlorinating PCE to ethene at the time. DNA was extracted from five separate one-gram samples of wet sediment (5.04 g total) using the Ultraclean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA). The manufacturer's protocol was followed, with the exception that a Mini-beadbeater™ (Biospec Products, Bartsville, OK) was used instead of a vortex for cell lysis. Procedures of DNA extraction and PCR reactions have been described previously (*8*).

PCR was performed in 20 μL reactions with 2.5x Eppendorf PCR Mastermix (Brinkmann, Westbury, NY), which contained 1.25 U Taq polymerase and 200 μM of each

deoxynucleoside triphosphate. For each reaction, 30 to 50 ng of template DNA was used. Nested PCR was performed using the universal bacterial primers in the initial PCR followed by a second PCR round with the dechlorinator 16S rRNA gene-targeted primer pairs (*13*). Chromosomal DNA from *Desulfuromonas michiganensis* strain BB1, *Desulfomonile tiedjei*, and *Geobacter lovleyi* strain SZ were used as positive controls. The amplicons were visualized on a 1% agarose gel in Tris-acetate-EDTA buffer and stained in an ethidium bromide solution. The primer sequences for the target 16S rRNA genes are provided elsewhere (*8*). Negative control reactions accompanied all PCR analyses and consisted of nuclease free water (Fisher Scientific).

Quantitative Real-Time PCR (qPCR). The number of *Dehalococcoides* and copies of reductive dehalogenase genes (RDases) *tceA*, *vcrA*, and *bvcA* were estimated using qPCR. The qPCR reactions for *Dehalococcoides* 16S rRNA genes and the *tceA*, *bvcA*, and *vcrA* genes were all performed as described by Ritalahti et al. (*14*). Standard curves for qPCR were prepared with a dilution series of quantified genomic DNA from *Dehalococcoides* sp. strain FL2 or from plasmids carrying a single *Dehalococcoides* RDase gene. The linear range for quantification was $10^2 - 10^{10}$ gene copies·mL^{-1} ($r^2 = 0.999$) for *Dehalococcoides* sp. strain FL2. The linear range for the target RDases was $10^2 - 10^{10}$ gene copies·mL^{-1} ($r^2 = 0.995$), $10^1 - 10^{10}$ gene copies·mL^{-1} ($r^2 = 0.997$), and $10^1 - 10^8$ gene copies·mL^{-1} ($r^2 = 0.990$) for *tceA*, *vcrA*, and *bcvA*, respectively. The gene copy numbers were calculated as described previously (*14*). Genome and genomic analyses show that the 16S rRNA and the RDase genes exist as single copy genes on *Dehalococcoides* genomes (*15-17*).

Sediment Column. A 1-D upflow sediment column was assembled to simulate an anaerobic sediment bed modified with an *in situ* cap. Exogenous sources of electron donor were not provided and the column was operated under relatively fast flow rates. The column was constructed using a 2.5 cm I.D. glass chromatography column 30 cm in length (Spectrum Chromatography, Houston, TX) with custom-built stainless steel end plates (Dutton & Hall, Inc., Atlanta, GA). A 2.5 cm diameter disc of stainless steel 80 mesh (Small Parts, Inc., Miami Lakes, FL) and a second 2.5 cm diameter disc of finer mesh (5 μM opening; TWP Inc., Berkeley, CA) were placed in series within the end plates to help distribute flow and prevent the loss of sediment particles. A glass reservoir fitted with a stopcock was fabricated and placed at the column effluent to allow for aqueous sampling. The column was packed with wet, homogenized sediment in a fume hood under a constant stream of N_2 and wrapped in foil. Estimates of porosity and water content were obtained from bulk sediment properties (Table 1) and column mass difference before and after packing.

Column influent composed of simulated groundwater spiked with aqueous PCE (ranging from 31.4 ± 11.3 μM to 134.7 ± 47.1 μM; Figure 5B) was supplied to the column by a syringe pump at a rate of 5.4675 mL·hr^{-1} to produce a hydraulic residence time of one day and a seepage velocity of 46.5 cm·day^{-1} (Darcy velocity = 25.8 cm·day^{-1}). The simulated groundwater was autoclaved at 121° C for 30 minutes and sparged with N_2 for 45 minutes prior to use. Periodic measurements of the influent indicated that dissolved oxygen levels were routinely below 0.5 mg·L^{-1} and always below 1 mg·L^{-1} (data not shown). The pH of the sparged groundwater was adjusted to circumneutral by the addition of carbon dioxide gas. Aqueous phase PCE, obtained from a saturated stock solution of neat PCE in anaerobic, sterile simulated groundwater, was added to the influent syringe immediately preceding connection to influent tubing. The column lifetime was divided into three separate phases based on influent conditions: Phase I from 0 to 108 pore volumes; Phase II from 108 to 146 pore volumes; and Phase III from 146 pore volumes

until the conclusion of the experiment. Phase I was defined by an influent composed of only simulated groundwater and PCE with the goal of obtaining baseline transport and biotransformation data. Phase II was characterized by the addition of 5 mM lactate to the influent from an anaerobic, sterile stock solution intended to stimulate the microbial community. In Phase III, the influent was returned to the initial Phase I conditions, containing only simulated groundwater and aqueous phase PCE.

Influent concentrations of PCE were routinely measured due to variability in the concentration of the aqueous PCE stock and to ensure that no PCE transformations occurred prior to introduction into the column. Aqueous effluent samples (5 mL) were obtained from the sampling reservoir and transferred to a sealed serum bottle that had an equivalent amount of gas-phase removed immediately prior to injection. Chlorinated ethenes, ethene, ethane, methane, and hydrogen concentrations were determined from headspace samples of these serum bottles. Aqueous samples (1 mL) were obtained from the serum bottles to measure effluent anion concentrations. Colorimetric iron analysis was performed on reservoir samples transferred directly to cuvettes. Two tracer studies were conducted, both during Phase I of the column: from 5 to 8 pore volumes and from 96 to 101 pore volumes. Bromide was supplied to the influent at a concentration of 100 $mg \cdot L^{-1}$ (1.25 mM) from an anaerobic, sterile stock of potassium bromide in simulated groundwater. A fraction collector captured tracer effluent in 4 mL increments and bromide was quantified via ion chromatography. Dechlorination, methanogenesis, and hydrogen production were not measured in the column effluent during the fraction collecting.

Sand Column Operation. Two 1-D upflow sand columns (designated herein as Sand Column A and Sand Column B) were constructed using 2.5 cm I.D. glass chromatography columns 30 cm in length (Spectrum Chromatography, Houston, TX) with custom-built stainless steel end plates (Dutton & Hall, Atlanta, GA). A 2.5 cm diameter disc of stainless steel 80 mesh (Small Parts, Inc., Miami Lakes, FL) was placed within the column end plates to help distribute flow and to prevent loss of sand particles from the column. Stainless steel was used for all fittings and tubing. A glass reservoir fitted with a stopcock was fabricated and placed at the column effluent to allow for aqueous effluent sampling. The columns were packed with C-33 concrete sand obtained from U.S. Silica (Mauricetown, NJ). The sand grade was selected due to its employment as a traditional cap in the Anacostia River Capping Demonstration Project performed by the Hazardous Substance Research Center – South and Southwest (*18*). The sand has a heterogeneous particle size distribution ranging from 0.15 to 4.75 mm in diameter and is composed mostly of medium to large sized particles and pebbles. The sand was used as received with the exception of autoclaving prior to application.

The dry, autoclaved sand was packed into both columns under aerobic conditions and mixed every 5-cm by vibration along the outer wall. Downward pressure was applied to the top of the sand during mixing to prevent preferential settling of larger particles toward the outer region of the columns. Following column packing, nitrogen gas was flushed through the columns for 15 minutes to remove oxygen, followed by carbon dioxide gas for 15 minutes to allow for complete dissolution of the gas phase within the columns. Sparged, autoclaved simulated groundwater was then passed through the columns to check for leakage. Measurement of saturated column masses provided estimates of porosity and water content for each column. A recently characterized PCE to ethene dechlorinating mixed consortia (referred to as "Owls" in (*8*)) served as the inoculum. An aliquot of Owls culture was sparged for 15 minutes, introduced into the columns through the influent, and allowed to remain in the columns for 24 hours to promote attachment. Both sand columns were wrapped in foil to avoid exposure to light. Table

5 provides a summary of experimental conditions for each column, with each column described in more detail below.

Sand Column A. The void volume for Sand Column A was estimated to be 62.72 mL based on mass difference and assuming complete water saturation. Prior to inoculating the column, a tracer test was conducted with 100 $mg \cdot L^{-1}$ (1.25 mM) bromide obtained from an autoclaved, sparged stock solution of potassium bromide in simulated groundwater. A total of 1.16 pore volumes were flushed through the column, collected with a fraction collector, and analyzed via ion chromatography. The Owls mixed culture used for the inoculum was enriched from contaminated groundwater site in Texas. It is maintained in a large reservoir and provided PCE, reduced mineral media, and methanol daily (*8*). A 200 mL aliquot of aqueous culture was obtained and stored in a 160 mL serum bottle that had previously been capped with a teflon-faced butyl septum and sparged with nitrogen for 15 minutes to remove oxygen. The 200 mL aliquot was tested for its dechlorination ability in batch conditions by spiking with PCE and methanol. After successfully dechlorinating PCE to ethene, 1.48 pore volumes were fed to the column at a flow rate of 2.163 $mL \cdot hr^{-1}$ (1-day residence time). Following the 24-hour attachment period during which there was no flow, Sand Column A was emplaced in series with the sediment column from 67 to 83 sediment pore volumes (see Chapter 2). A schematic of the laboratory set-up is shown in Figure 7. The unamended sediment column effluent served as the influent for the duration of the Sand Column A experiment. The effluent product distribution from Sand Column A therefore reflects the capacity of sediment effluent to maintain an external dechlorinating community, in this case simulating a bioreactive cap inoculated with a mixed dechlorinating culture and operating under reducing conditions.

Sand Column B. The Sand Column B experiment was also designed to simulate a dechlorinating bioreactive cap operating under reducing conditions, but for this experiment the influent was spiked with electron donor. The void volume for Sand Column B was estimated to be 61.82 mL based on mass difference and assuming complete water-saturation. A tracer test was not performed on this column, however, due to time constraints. An aliquot of Owls culture was retrieved and sparged with nitrogen prior to inoculation as described above for Sand Column A. The aliquot of Owls culture again demonstrated the ability to completely dechlorinate PCE to ethene in batch culture. A total of 1.65 pore volumes of Owls culture were then supplied to the column at a flow rate of 2.5758 $mL \cdot hr^{-1}$ (1-day residence time). Sand Column B was not immediately connected to the sediment column effluent in order to perform positive-control experiments ensuring the column could dechlorinate *cis*-DCE when provided media, vitamins, electron donor, and a carbon source. Following this demonstration of complete dechlorination, sediment column effluent was obtained from 146 to 180 pore volumes (see Chapter 2) to serve as Sand Column B influent. Sediment column effluent was captured anaerobically by connecting an empty, gas-tight syringe to sediment effluent tubing and allowing the aqueous flow to gradually fill the syringe at the same rate of sediment column influent (5.4675 $mL \cdot hr^{-1}$; Chapter 2). Once the effluent syringe had been filled, it was transferred to a separate syringe pump and introduced into the sand column as the influent. This method allowed for manipulation of flow rates and for addition of electron donor to the influent prior to connection with the sand column. The electron donor used for this study was lactate, which was obtained from a 100 mM stock solution in autoclaved, sparged simulated groundwater. Lactate was supplied to the influent at a concentration of 5 mM from 0 to 13.27 sand pore volumes. Aqueous *cis*-DCE was obtained from a saturated stock solution (NAPL present) of *cis*-DCE in autoclaved, sparged simulated groundwater and supplied to the influent at a concentration of 200 ± 42 μM from 0 to 3.44 sand pore volumes.

The experimental conditions of Sand Column B were designed to gradually decrease aqueous residence times as well as electron donor concentrations to determine limitations on dechlorination. The experimental conditions are illustrated graphically in Figure 9A which displays the different flow rates and concentrations of lactate employed, and Table 5 provides a summary. The influent flow rate for Sand Column B was increased step-wise from 1.2879 $mL \cdot hr^{-1}$ (2-day retention time), to 2.5758 $mL \cdot hr^{-1}$ (1-day retention time) to 5.4675 $mL \cdot hr^{-1}$ (0.47-day retention time). The flow rate of 5.4675 $mL \cdot hr^{-1}$ corresponds to the flow rate exiting the sediment column (Chapter 2). From 0 to 3.44 sand pore volumes, additional *cis*-DCE was provided to the influent to ensure chlorinated ethenes were present (complete dechlorination was observed in the sediment column effluent prior to connecting Sand Column B). After 3.44 pore volumes, the only source of chloroethenes was sediment effluent. Lactate was provided from 0 to 13.27 pore volumes, at which point it was removed from the influent. The effluent product distribution from Sand Column B should reflect the impact of flow rate and the presence of reducing equivalents on the capacity of the sediment column effluent to maintain an external dechlorinating community.

Analytical Methods. Chloroethenes, ethene, ethane, and methane were separated via gas chromatography (GC) and analyzed with an FID detector, as described previously (*19*). Hydrogen concentrations were determined from headspace samples using a GC equipped with a reducing gas analyzer (*20*). Iron measurements were conducted using a modified ferrozine method (*21*) with colorimetric determination of ferrous and ferric iron by a Cary 300 UV-VIS spectrophotometer. Anions were separated using a Dionex DX-100 ion chromatograph with a Dionex AG4A IonPac guard column and Dionex AS4A IonPac column at a flow rate of 1.5 $mL \cdot min^{-1}$ and analyzed using an ED40 electrochemical detector.

II. Results

Sediment characterization. The results of the sediment characterization are summarized in Table 1. The values for wet bulk density, particle density, and gravimetric water content were primarily used to estimate the masses and volumes of solid, aqueous, and gas phases during microcosm and column construction. The results of the particle size analysis show that the majority of the sediment is composed of silts and sands according to the Wentworth classification system (*22*). Mass recovery from the sieves during particle size analysis averaged 95.5% ± .086%. The measured fraction organic carbon was measured to be 5.22%, suggesting that carbon is available for indigenous microbes. The organic carbon measurement was also used for estimating K_D values for the chlorinated solvents and for obtaining an observed K_{oc} value for PCE. A measured salinity of 0.25 ppt was close to a reported value of 0.29 ppt (*1*).

PCE Sorption. The results of the abiotic PCE microcosms are shown in Figure 1, with the observed aqueous concentrations normalized to the initial PCE mass introduced. Neither dechlorination nor methanogenic activity was observed in the autoclaved microcosms, indicating adequate sediment sterilization. Abiotic transformations of PCE were also not observed. The disappearance of PCE over time is therefore attributed to sorption to the solid-phase of the sediment. Modeled equilibrium aqueous phase PCE concentration was calculated to be 0.178 (shown as a dashed line in Figure 1) based on partitioning of PCE among the gas, aqueous, and solid phases and the known mass of PCE added measured. Equilibrium PCE sorption showed good agreement with the estimated value after 41 days, and no further disappearance of PCE

occurred after this time (Figure 1). The measured K_{oc} value after 41 days was calculated from the change in aqueous phase concentration and found to be 312 $mL \cdot g^{-1}$, which is similar to the reference value of 265 $mL \cdot g^{-1}$ (*3*). The fitted sorption rate coefficient (k) for the nonequilibrium sorption model was calculated to be -5.48 x 10^{-3} hr^{-1}, as shown in Figure 1.

Microcosms

PCE Microcosms. PCE was successfully dechlorinated to the end products of ethene and ethane under methanogenic conditions in all biologically active microcosms. Figure 2 shows a plot of a representative microcosm following the three initial PCE dechlorination cycles. This microcosm was supplied with acetate and hydrogen, resulting in 1.3 mequiv of electrons (electron donor to PCE ratio = 31.3). Temporary stalling of dechlorination at *cis*-DCE occurred during the first dechlorination cycle in most microcosms. The loss of ethene mass balance during the first spike was attributed to leakage through the septa after multiple punctures, as evidenced by the loss of methane after approximately 40 days. During the subsequent dechlorination cycles, rapid dechlorination occurred and ethane production was observed. Interestingly, a concurrent increase in methanogenesis was observed during conversion of ethene to ethane, implicating the role of methanogens in this process (*23, 24*).

The effect of exogenous electron donor supply on the time required for complete dechlorination is presented in Table 2, along with the amount of reducing equivalents provided. It is generally observed that increasing amounts of reducing equivalents (i.e., increasing the electron donor to PCE ratio) decreased the time necessary for complete conversion of PCE to ethene, especially after the second and third PCE additions. Notably, complete dechlorination was observed in microcosms not provided with additional reducing equivalents. These results are in agreement with previous studies reporting that in the absence of nutrient and substrate amendments, sediment can serve as a nutrient source with the system as a whole being electron donor limited (*25, 26*). It appears that supplying fermentable substrates may enhance reductive dechlorination in field sediments. This technique of biostimulation by supplying electron donors is widely applied in aquifer remediation scenarios (*27, 28*). The bioaugmented control microcosm did not perform substantially better than other microorganisms after the initial dechlorination cycle, suggesting the non-bioaugmented microcosms were not limited by cell numbers. PCE transformation was not observed in the autoclaved control microcosm, and its disappearance over time was attributed to sorption. Taken together, these results suggest that the natural attenuation of PCE via reductive dechlorination appears feasible by Anacostia River sediment under static conditions.

DCE Screening Cultures. The ability of a culture enriched from Anacostia sediment on PCE to dechlorinate DCE isomers other than *cis*-DCE was investigated with the results shown in Figures 3A–C. Aliquots of culture successfully dechlorinated 1,1-DCE to ethene under methanogenic conditions, but were unable to dechlorinate *trans*-DCE. The culture is normally exposed to *cis*-DCE during its dechlorination of PCE, with alternative DCE isomers generally not detected. The ability of the mixed culture to utilize 1,1-DCE, but not *trans*-DCE, as an electron acceptor may implicate certain microorganisms in dechlorination past DCEs (see discussion under *Microbial Analysis* heading below), and may limit the sediment's capacity to naturally bioattenuate chlorinated ethene plumes, considering that 39% of current or former EPA NPL sites contain *trans*-DCE (*29*). Methane production in the 1,1-DCE and *trans*-DCE cultures were similar, but a positive control culture fed PCE appeared to stifle methanogenesis slightly. This decrease in methane production was also observed in the active PCE microcosms during

each sequential dechlorination cycle, shown in Figure 2. PCE inhibition of methanogenesis has been reported by previous researchers and may be causing this response (*30-32*).

VC Oxidation Microcosms. A representative graph of potential VC oxidation microcosms is shown in Figure 4. Vinyl chloride disappears from the aerobic microcosm without the production of ethene nor the loss of methanogenesis. The disappearance is attributed to vinyl chloride oxidation to CO_2, which was not measured. The utilization of VC as the sole carbon and energy source has been reported for *Mycobacterium* and *Pseudomonas* isolated from soil, river water, and activated sludge (*33-36*). VC can also be oxidized to CO_2 through cometabolic processes in the presence of monoxygenase inducers, such as methane (*37*), ethane (*38*), ethene (*39*), and propane (*40*), among others. The VC loss observed does not coincide with methane loss, suggesting that methane is not inducing cometabolic oxidation of VC, but further research needs to be completed to draw any steadfast conclusions.

Microbial Analysis. Results of the microbial analysis are summarized in Table 3. A number of dechlorinating populations were detected in Anacostia River sediment. *Dehalobacter restrictus*, *Desulfuromonas* species, and *Desulfomonile* species were present in the sediment, all of which are capable of dechlorinating PCE to DCE. Two *Dehalobacter restictus* strains, PER-K23 (*41*) and TEA (*42*), have been isolated, characterized, and are detected with the primers employed. The *Dehalobacter* strains are strictly hydrodenotrophic and have previously been detected in enrichment cultures originating from river sediments (*41, 43, 44*). *Dehalobacter restrictus* strain PER-K23 is completely dependent on chlorinated hydrocarbons as an electron acceptor and does not form spores (*45*). *Desulfuromonas sp.* strain BB1 and *Desulfuromonas chloroethenica* oxidize acetate, but not hydrogen, for the reductive dechlorination process (*46*). *Desulfuromonas* species have previously been detected in cultures enriched from river sediments (*46-48*). *Desulfomonile* species are capable using sulfate and halobenzoates as electron acceptors in addition to PCE, and can utilize a wide range of electron donors, including hydrogen (*49, 50*).

The sediment also contained *Dehalococcoides* species, which have the unique ability to conserve energy for growth during each step of the PCE dechlorination pathway. *Dehalococcoides* are also capable of dechlorinating organics other than chloroethenes, such as chlorinated benzenes, dioxins, and PCBs (*51-53*). *Dehalococcoides* was quantified at a concentration of $5.19 \times 10^8 \pm 2.25 \times 10^7$ cells·mL^{-1} from a culture fed PCE, acetate, and hydrogen. The target reductive dehalogenase genes *tceA*, *vcrA*, and *bvcA* were all amplified from the extracted DNA to yield concentrations provided in Table 3, with the *vcrA* gene being most abundant, followed by the *tceA* and *bvcA* genes, respectively. The amplification of the *vcrA* gene suggests the presence of *Dehalococcoides* sp. strain VS and/or GT. Strain VS can utilize TCE, *cis*-DCE, 1,1-DCE and VC for growth but has not yet been purified (*54*). Strain GT can couple VC dechlorination with growth and is also capable of dechlorinating TCE, *cis*-DCE, and 1,1-DCE for growth and contains the *vcrA* gene (*55*). The amplifiation of the *tceA* gene suggests at least one of the strains FL2 or 195 are present. *Dehalococcoides* sp. strain FL2 can utilize TCE, *cis*-DCE, and *trans*-DCE as electron acceptors and can cometabolize PCE and VC (*56*). *D. ethenogenes* sp. strain 195 can couple growth with the dechlorination PCE to VC, while cometabolizing VC to ethene for complete detoxification (*57*). Finally, the amplification of the *bvcA* gene suggests that a BAV1-like organism is present in the culture. Strain BAV1 can utilize a suite of halogenated electron acceptors including *cis*-DCE, 1,1-DCE, VC, vinyl bromide, and 1,2-dichlororethane while utilizing acetate as an electron donor (*58*). BAV1 can also cometabolize PCE and TCE in the presence of a growth supporting DCE isomer or VC.

Since the sediment-free PCE dechlorinating culture could successfully dechlorinate 1,1-DCE but not *trans*-DCE, the presence of BAV1 and FL2 is questionable given their ability to dechlorinate *trans*-DCE. DNA extracted from the source culture for the DCE screening experiments (sediment-free enrichment maintained within a 500 mL reactor) also amplified the *bvcA* gene, albeit at low concentrations (~10^4 gene copies·mL^{-1}; data not shown). It is possible that the BAV1 type microorganism was not growing in the source culture due to unfavorable culturing conditions or competition for electron acceptors by fellow *Dehalococcoides* strains. When transferred during the DCE isomer screening study, BAV1 therefore may not have been viable. *Dehalococcoides* sp. strains 195, VS, and GT are the only known strains that can reduce 1,1-DCE but not *trans*-DCE for growth. Further research on the DCE cultures needs to be conducted to resolve the incongruity of no *trans*-DCE dechlorination despite the apparent presence of BAV1.

Sediment Column. Results of the sediment column are presented in Figures 5A-G and Table 4. The experimental conditions applied to the column are illustrated in Figure 5A and show that a total of 180 pore volumes of simulated groundwater supplemented with aqueous PCE were flushed through the column with a 1-day retention time. The flow rate was selected to simulate potentially elevated seepage velocities associated with flow bypassing caused by *in situ* capping with relatively low permeability caps. The column seepage velocity of 47.0 cm·day^{-1} is on the upper end of the range of observed field velocities of groundwater seeps (0.0026 to 720 cm·day^{-1} (*59*), but not unrealistic for a capping scenario. Assuming a 10ft × 10ft area of sediment is subject to groundwater seepage at a mean Darcy velocity of 4.2 cm·day^{-1} (as reported for Anacostia River sediment (*1*)), and a low permeability cap diverts discharge to a localized zone of 1ft × 1ft, the reduction in discharge area would cause a 100 fold increase in specific discharge to 17.5 cm·hr^{-1}. This value is similar to our experimental specific discharge of 25.8 cm·hr^{-1}. Methanogenesis (Figure 5E), iron sulfide precipitation, and the elution of phosphate (Figure 5G) were observed shortly after flow was initiated, and are all indicators of reducing conditions within the column.

Phase I. The sorption and partial dechlorination of chloroethenes, along with the loss of methanogenesis, were observed during Phase I of the column experiment. The influent concentration of PCE was 31.40 ± 11.27 μM from the onset of flow to about 32 pore volumes (Figure 5B). At this point, the influent PCE concentration was intentionally increased to 61.20 ± 9.55 μM in order to promote chloroethene elution and to ensure that once dechlorination products did elute, they were quantifiable. The influent concentration was again increased at 62 pore volumes to examine the effect on increased PCE influent flux on effluent concentration and product distribution. No ethenes were observed in the effluent until 46 pore volumes, at which point *cis*-DCE was detected. Figure 5C shows the effluent concentrations of PCE and its dechlorination products, while a plot of normalized product distribution with pore volumes eluted is presented in Figure 5D. Examination of Figure 5C reveals that effluent *cis*-DCE molar concentrations were markedly below the corresponding influent PCE concentrations, attributed to sorption of chloroethenes by the solid phase. The appearance of PCE and TCE in the column effluent began at approximately 85 pore volumes (inset – Figure 5C), and was attributed to an increase of influent PCE concentrations (Figure 5B) combined with a loss of microbial activity, indicated by methane concentrations approaching non-detect levels beginning at 45 pore volumes (Figure 5E).

The cause for the scattering of chloroethene effluent concentrations is unknown, and assessment of column plumbing (e.g., leaks), sampling procedures (e.g., obtaining replicate

samples, increasing sampling frequency, changing sampling vial), and calibration curves yielded no experimental nor analytical reasons for the scattered data. One possibility is that dechlorinating activity within the sediment column was temporarily stimulated by localized breakdown of organic matter, resulting in desorption of chloroethenes and transient elevations in aqueous phase concentrations. This process of biologically enhanced desorption is analogous to enhanced dissolution of NAPL caused by microbial activity. Another potential cause for the concentration scattering is the non-ideal flow within the column, as revealed by the tracer studies. The results of the tracer studies are summarized in Table 4 and suggest extremely non-ideal flow patterns caused by physical nonequilibrium conditions (e.g., dead end pores). The flow conditions are a result of the naturally heterogeneous sediment and microbial gas generation. Perhaps the transient changes in effluent chloroethene concentrations were due to the formation of new flow pathways within the hydraulically dynamic sediment. Conant et al. (*60*) characterized a chloroethene plume discharging into a river and reported complex porewater concentration distributions over relatively small lateral distances (from <1 to 3.5 meters) due to the presence of preferential flow patterns.

Methane concentrations approached zero between 45 to 108 pore volumes (Figure 5E), indicating a loss of microbial activity within the sediment. The presence of reduced iron (Figure 5G) and the lack of dissolved oxygen (data not shown) in the effluent confirm that the sediment had maintained reducing conditions. It is possible that dechlorination and methanogenesis were outcompeted for energy during this period, due to the existence of ferric iron and sulfate reduction, causing the observed drop in these respective processes. The absence of hydrogen in the effluent during this period does not support this reasoning, however, since dechlorination and methanogenesis would become thermodynamically favorable at such low hydrogen concentrations. The decrease in methanogenesis could also have been caused by constant exposure to higher chlorinated ethenes, which were shown to inhibit methanogens during the PCE and DCE microcosm experiments (Figure 2; Figures 3A-C). This indeed may have caused some *Archaea* to be inhibited, but it would also be expected that dechlorination would still proceed, especially considering that a greater share of reducing equivalents would become available to the dechlorinators following the loss of methanogens. Instead it was hypothesized that the loss of dechlorination and methanogenesis was due to a lack of available electron donor, and the addition of lactate to the influent was initiated to test this hypothesis.

Phase II. The addition of 5 mM lactate during pore volumes 108 to 146 stimulated the microbial community, evidenced by the revival of dechlorinating and methanogenic activities and a spike in hydrogen concentrations. Effluent chloroethene distribution shifted from a mix of PCE, TCE, and *cis*-DCE prior to lactate addition to solely *cis*-DCE almost immediately following (Figure 5D). Total chloroethene effluent concentrations during Phase II when *cis*-DCE was the predominant product (i.e., from pore volume 111 to 124) averaged 68.6 ± 22.0 μM, similar to influent PCE concentrations of 68.9 ± 16.7 μM during this period. The appearance of VC and ethene began at approximately 118 and 124 pore volumes, respectively, with ethene eventually becoming the dominant product (Figure 5D). Interestingly, ethene levels then increased to concentrations greater than the influent PCE concentration. Total chloroethene effluent concentrations between pore volumes 127 to 147 averaged 172.2 ± 34.3 μM, which was roughly 2.5 times greater than influent concentrations. The increase in dechlorinating activity is accompanied by an increase in chloride at 144 pore volumes (Figure 5F).

It is believed that biologically enhanced desorption contributed to the increased total chloroethene effluent concentrations observed during this phase of the column study. Additionally, dechlorination leads to increasingly less sorptive products (i.e., lower K_{oc} values),

which allows for greater aqueous phase concentrations of ethenes, which also probably contributed to the elevated chloroethene concentrations. The use of relatively high flow rates enhanced this process by maintaining a large difference between actual and equilibrium chloroethene aqueous phase concentrations. The removal of electron donor from the influent during Phase III appeared to cause chloroethene levels to return to influent concentrations (Figure 5C – see discussion below), suggesting that stimulation of the microbial community caused the elevated chloroethene concentrations. This observation may have ramifications for historically contaminated sites with large amounts of sorbed mass that are to be treated using biologically based remediation. Dechlorination of previously sorbed chloroethene mass may occur during the onset of microbial stimulation, and indeed has been implied (*27*).

An effluent sample obtained during Phase II was used as the inoculum for a microcosm containing DCB-1 media supplemented with vitamins, lactate (5 mM), and hydrogen (20 % headspace). PCE and VC were added to test for dechlorinating activity within the microcosm. Both PCE and VC were both chosen as electron acceptors to allow activity of all dechlorinating organisms (i.e., PCE to DCE species and DCE to ethene species). The dechlorination of both PCE and VC were observed after a brief lag period (Figure 6A). The presence of ethene in the microcosm indicates the presence of *Dehalococcoides*. The washout of viable dechlorinating organisms, specifically *Dehalococcoides*, suggests that *in situ* caps may become colonized by microbial species indigenous to the underlying bulk sediment. This process may enhance the efficacy of bioreactive *in situ* caps and support their successful deployment.

The stimulation of the non-dechlorinating microbial community during Phase II is supported by the increase in effluent concentrations of methane, hydrogen, and ferrous iron. Methane concentrations approached non-detect levels prior to lactate addition, but were returning to initial levels at the conclusion of Phase II (Figure 5D). Figure 5E shows the effluent hydrogen concentrations at non-detect during Phase I and significantly elevated during Phase II. A substantial hydrogen peak was observed at 142 pore volumes, almost 3 orders-of-magnitude greater than levels associated with minimum hydrogen thresholds for dechlorinators (*20, 61*), suggesting that much of the hydrogen being produced was eluting from of the sediment column. Lactate is fermented to propionate and acetate while releasing 4 reducing equlivalents in the form of molecular hydrogen. A modest spike in ferrous iron at approximately 142 pore volumes (Figure 5F) indicates that iron-reducing bacteria were also stimulated within the sediment by lactate addition. Although the addition of electron donor successfully stimulated the microbial community within the sediment, it was unclear whether this activity would be sustained after lactate addition ceased. It was hypothesized that the fermentation of organic matter would maintain the observed VC and ethene production during Phase II, and this was tested during Phase III by removing lactate from the influent.

Phase III. The lack of electron donor during Phase III caused an immediate decrease in microbial activity, demonstrated by simultaneous reductions of dechlorinating activity, methanogenesis, and hydrogen production. Chloroethene product distributions shifted from complete dechlorination to a mixture of *cis*-DCE, VC, and ethene for the remainder of the experiment. Total chloroethene concentrations decreased from the end of Phase II (172.2 ± 34.3 μM) to the beginning of Phase III (67.9 ± 10.8 μM), during which period it was similar to influent concentrations (68.9 ± 16.7 μM). It appears that the lack of electron donor interrupted the proposed enhanced desorption process discussed in Phase II. This is supported by effluent methane concentrations, which exhibited a marked decrease immediately after the cessation of lactate addition, approaching non-detect despite the growth observed during Phase II. Hydrogen production also declined to non-detect levels, despite an observed peak at 154 pore volumes.

Ferrous iron and chloride levels returned to previous concentrations, without any significant spikes. An effluent sample was again retrieved and used as the inoculum to test dechlorinating activity as described earlier in Phase II. Figure 6B shows dechlorination was again observed, but with a longer lag period compared to the Phase II microcosm, qualitatively suggesting fewer viable cells were being washed out during Phase III.

The results of the sediment column experiment demonstrate that aquatic sediments serving as natural biobarriers for the detoxification of chlorinated solvents plumes may be limited by electron donor availability, especially when subject to elevated flow rates. This conclusion is supported by microcosm studies which also suggested that microbial activity was electron donor limited. These results are surprising given the relatively large amount of electron donor present in sediments in the form of organic matter and given previous field studies reporting chloroethene plume detoxification (*6, 60, 62-64*). Reducing equivalents from the oxidation of organic matter apparently were not available for microbes in this instance, perhaps due to the combined effects of organic matter recalcitrance, electron donor washout, and preferential flow path formation. In natural settings labile organic matter is quickly oxidized at the sediment-water interface, leaving more refractory organic matter subject to burial (*65*). This process may have occurred in the sediment column as a whole, since the sediment was homogenized and labile organic matter was evenly distributed. In addition, the relatively high flow rate used during the experiment could have forced the elution of fermentation products from the column, thus removing electron donor from the system prior to consumption. This is supported by the large hydrogen peak observed during Phase II. Finally, the presence of preferential flow paths may have eliminated some regions of the sediment from contacting the chloroethenes, effectively decreasing the amount of reducing equivalents available for dechlorinating microbes in the vicinity of the contaminants.

These findings have significant relevance to *in situ* capping scenarios where contaminant groundwater plumes are present. The loss of sedimentary bioattenuation processes observed in these studies suggests that groundwater contamination may travel through sediments without significant biotransformation and discharge into *in situ* caps and surface waters. Therefore, it appears that *in situ* caps should be designed to account for the loss of natural biobarrier reactivity and for the breakthrough of groundwater contaminants. The use of reactive caps during capping, whether physicochemical or biologically based, may be a viable solution.

Sand Column A. The normalized chloroethene effluent product distribution of Sand Column A is shown in Figure 8B. The 5.4675 $mL \cdot hr^{-1}$ flow rate of the column corresponded to a residence time of 0.47 days and a seepage velocity of 62.67 $cm \cdot day^{-1}$. During the time period when Sand Column A was connected in series to the sediment column, cis-DCE was the predominant product in the sediment effluent (see Figure 6C), and thus was the predominant chloroethene in the sand column influent. The sand column initially was able to dechlorinate cis-DCE to VC, but ethene was not detected (Figure 8B). This reactivity quickly disappeared prior to 5 pore volumes, and a mix of VC and cis-DCE was observed in the effluent for the remainder of Sand Column A. Eventually, only 5% of the cis-DCE was being dechlorinated to VC, indicating that Dehalococcoides activity was being impaired. Methane data collected during Sand Column A reveal that microbes other than dechlorinators also lost activity, suggesting a limitation in the system as a whole and not just for the dechlorinators (data not shown).

Based on Figure 8B, it appears as though sediment column effluent could not sustain a dechlorinating culture without additional amendments. Previously presented research (Chapter 2) demonstrated that microbial activity in the sediment column was limited by electron donor

availability. It was hypothesized that a lack of electron donor in the sediment column effluent prevented the dechlorinating community in the sand cap from being fully active. It was also suggested that the high flow rates through the sand column precluded complete dechlorination by not allowing sufficient contact time between the contaminants and the dechlorinating community. These hypotheses were addressed during Sand Column B operation.

Sand Column B. The experimental conditions associated with Sand Column B are presented in Figure 9A, with the chloroethene product distribution displayed by Figure 9B. Prior to feeding with sediment effluent, the column was fed cis-DCE along with lactate, reduced DCB-1 media, and vitamins in order to confirm the culture's ability to achieve complete dechlorination within the column. Complete dechlorination was observed during this period (data not shown), and sediment effluent was then fed to Sand Column B beginning at pore volume 0 as shown in Figures 9A-B. The influent for Sand Column B was the sediment effluent from 146 to 180 sediment pore volumes, and composed of a mixture of cis-DCE, VC, and ethene (see Figure 6C). Sand Column B was initially provided with cis-DCE and lactate while operating under a residence time of 2 days. Incomplete dechlorination was observed during this period, with a mix of VC and ethene in the sand column effluent. From 3.44 to 5.74 sand pore volumes, lactate was provided to the influent with the sediment effluent serving as the sole source of chloroethenes. The sand column successfully achieved complete dechlorination to ethene during this period, demonstrating that the sand cap could detoxify the flux of chloroethenes exiting the sediment column. This result, coupled with the lack of complete dechlorination during the previous condition (0 to 3.44 sand pore volumes) when additional cis-DCE was provided to the influent, suggests that chloroethene concentrations entering the sand column can limit the extent of dechlorination.

Figure 8B also suggests that electron donor concentrations within the cap may have an impact on dechlorination. Complete dechlorination was observed under fast flow rates (0.47 residence time) between 8.00 and 13.27 pore volumes when lactate was provided to the sand column. When lactate was removed from the influent at 13.27 pore volumes, however, a mixture of chloroethenes was observed in the effluent, indicating the importance of supplying external reducing equivalents to the sand column. In general, the results of Sand Column B suggest that influent chloroethene flux and electron donor availability impact the extent of dechlorination within a sand cap. Therefore, a successful reducing bioreactive in situ cap appears to require the incorporation of electron donor to maintain the dechlorinating community, and sufficient contaminant residence times to allow the dechlorinators time to biotransform high contaminant fluxes.

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IV. Figures and Tables

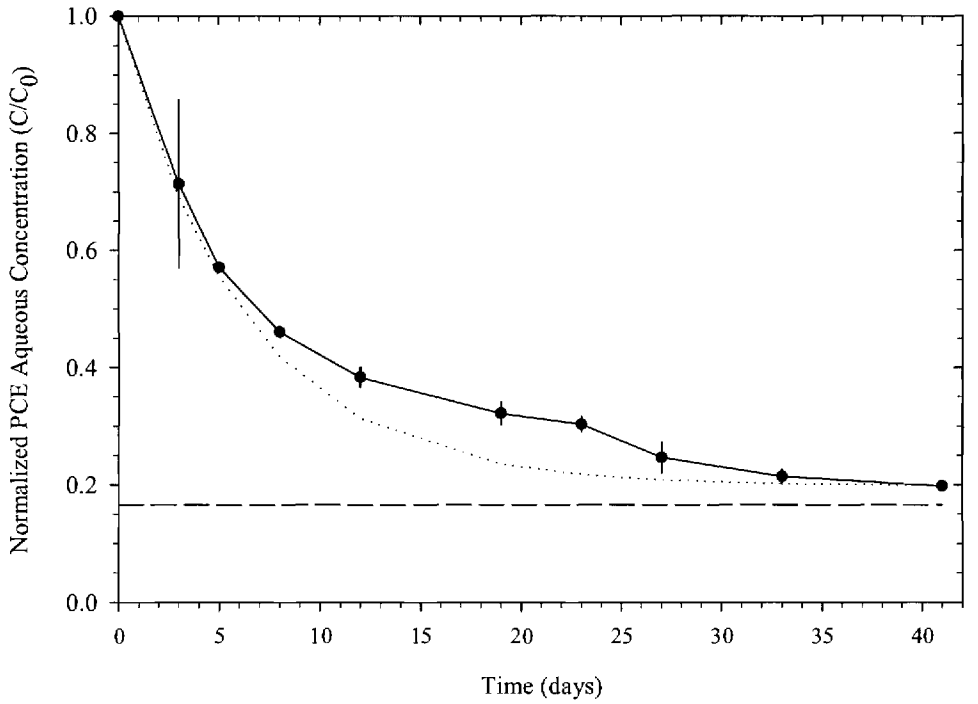


Figure 1. Aqueous phase concentration of PCE in sterilized microcosms normalized to initial PCE mass introduced. Measured values of aqueous phase PCE concentration are the average of duplicates, and error bars are one standard deviation. The dashed line is an estimate of equilibrium concentration of PCE based on partitioning among gas, aqueous, and solid phases. The dotted line is a one-site chemical nonequilibrium model fitted with $k = -5.48 \times 10^{-3}$ hr^{-1}.

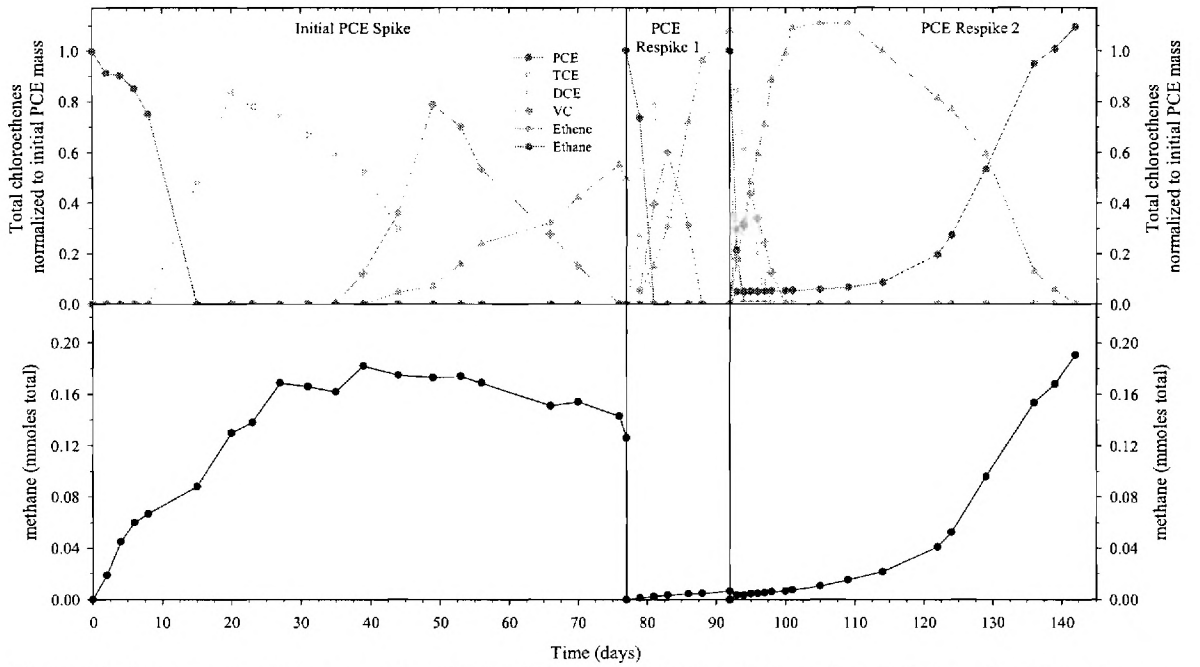
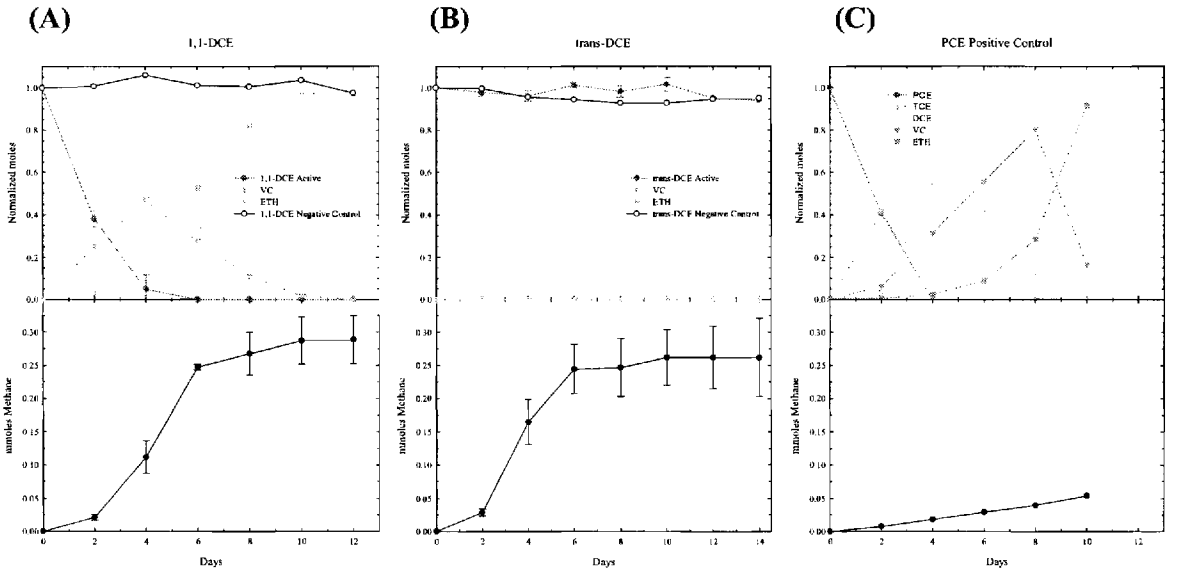


Figure 2. Representative example of initial three dechlorination cycles and associated methane production for active PCE microcosms. Chloroethene concentrations are plotted as the total mmoles of chloroethene species present in the microcosm (i.e., sum of solid, aqueous, and gas phases) normalized to initial mmoles of PCE introduced into the microcosm. Black vertical lines represent microcosm sparging and respike with PCE. Methane concentrations are plotted as total mmoles within the microcosm (i.e., sum of solid, aqueous, and gas phases). The example microcosm shown was provided 5 mM acetate and 3.5 % H_2 in microcosm headspace, resulting in an electron donor to PCE ratio of 16.6.



Figures 3A-C. Reductive dechlorination of DCE isomers and PCE with corresponding methane production by a PCE enrichment culture. (A) 1,1-DCE was dechlorinated to ethene while (B) *trans*-DCE was not dechlorinated to any extent. (C) The positive control dechlorinated PCE to ethene. Error bars indicate one standard deviation of duplicates.

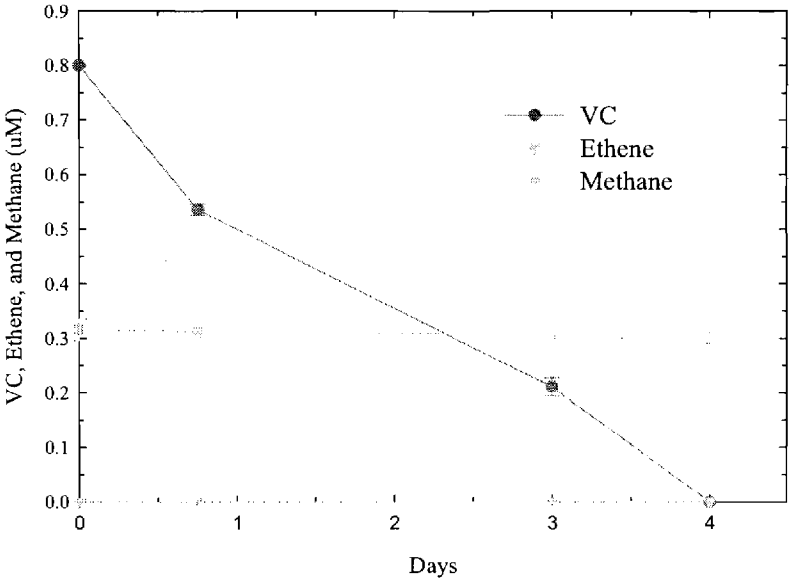
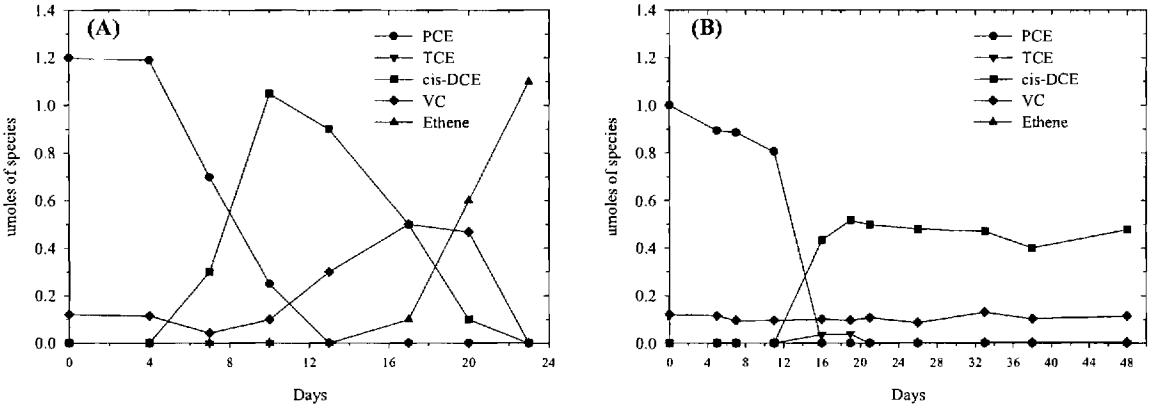


Figure 4. Representative plot of VC oxidation in sediment microcosms. Data points are the average of duplicates, with error bars representing one standard deviation.



Figures 6A-B: Dechlorination of PCE and VC in a microcosm inoculated with an aqueous sample of sediment column effluent obtained during (A) Phase II and (B) Phase III. The microcosm contained DCB-1 media supplemented with Wolin vitamins, lactate (5 mM), and hydrogen (20 % headspace). Both PCE and VC were added to the microcosm as electron acceptors to support the activity of all dechlorinating organisms (i.e., PCE to DCE species and VC to ethene species). Concentrations of dechlorination products are plotted as μmoles of species total (sum of aqueous and gas phases).

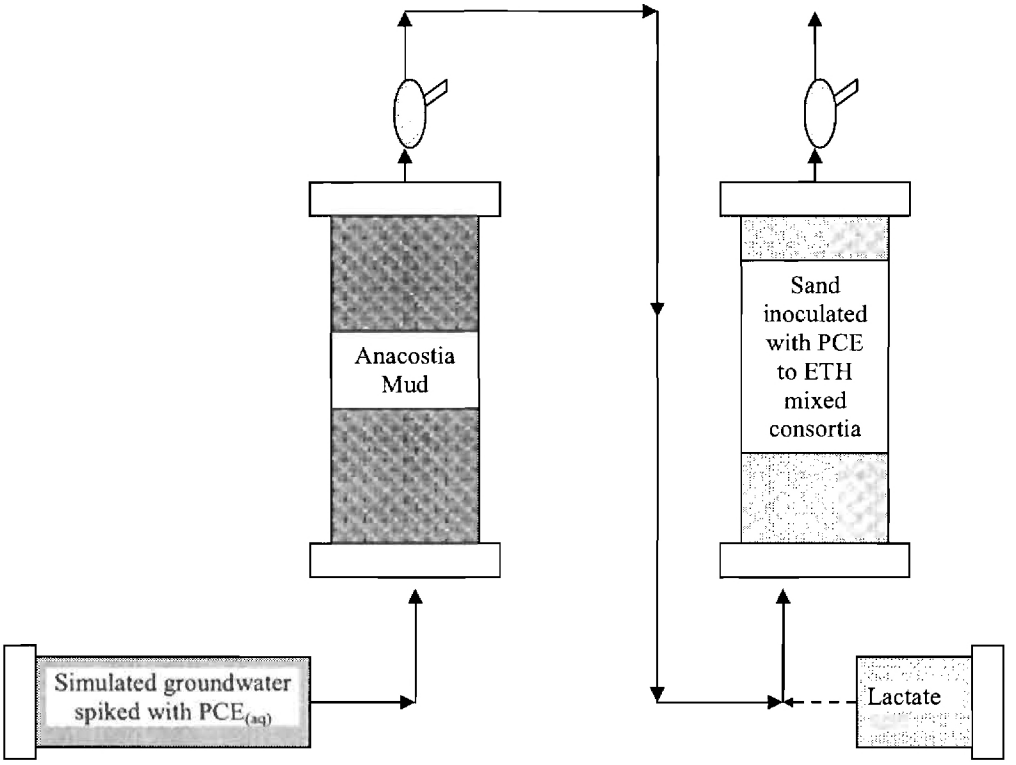
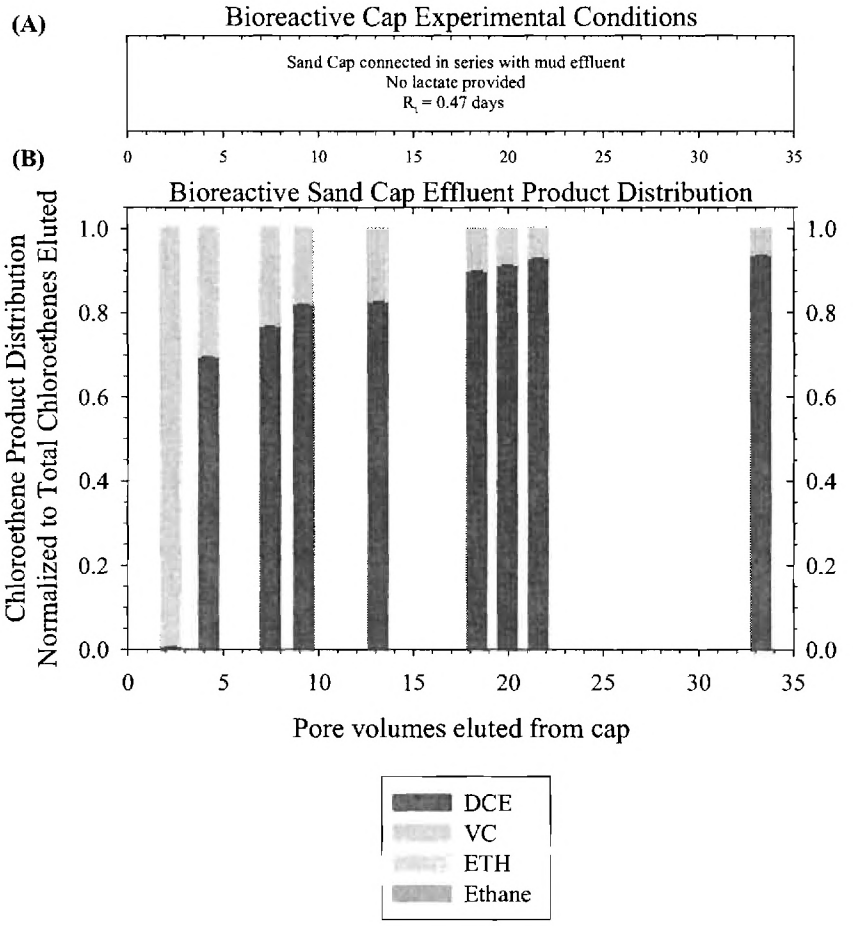
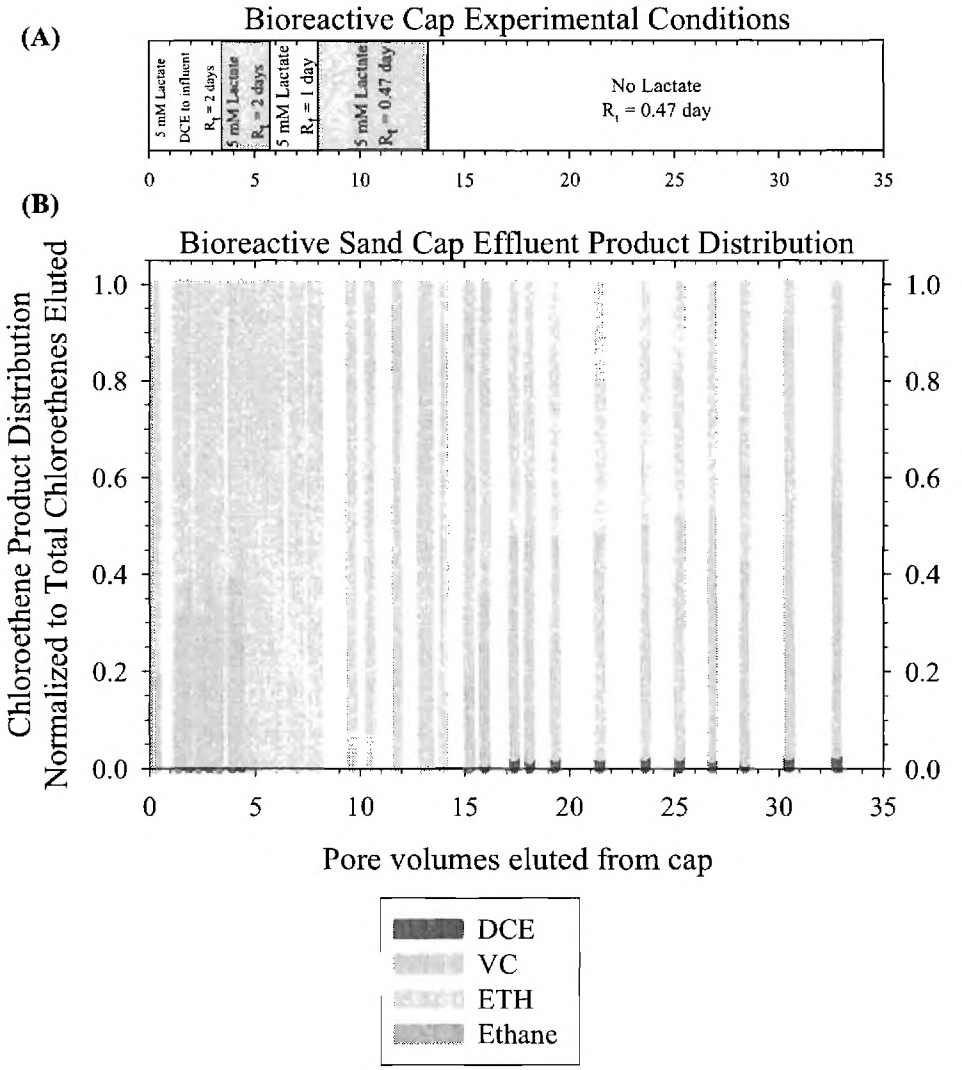


Figure 7. Schematic of laboratory simulation of a bioreactive sand cap placed in series with an anaerobic sediment bed subject to a PCE contaminated groundwater seep. Lactate was only added to the influent of Sand Column B from 0 to 13.27 pore volumes.



Figures 8A-B. (A) Operating conditions for Sand Column A during its lifetime. The effluent of the sediment column served as the influent of the sand column and was not amended with exogenous electron donors, electron acceptors, carbon sources, minerals, nor vitamins. (B) Effluent product distribution of Sand Column A inoculated with a PCE to ethene dechlorinating mixed consortia and connected in series with sediment column effluent between 68 and 83 sediment pore volumes.



Figures 9A-B. (A) Operating conditions for Sand Column B during its lifetime. The effluent of the sediment column served as the influent of the sand column and was not amended with minerals nor vitamins. Exogenous electron donor (lactate) , carbon sources (lactate) , and electron acceptor (cis-DCE) were added where indicated. (B) Effluent product distribution of Sand Column B inoculated with a PCE to ethene mixed dechlorinating consortia and connected in series with sediment column effluent between 146 and 185 sediment pore volumes.

Table 1. Results of the sediment characterization are summarized in Table 1. The particle size analysis was classified according to the Wentworth classification system (*22*).

Sediment Property	**Measured Value**
Wet bulk density	1.36 g/mL
Particle density	2.44 g/mL
Gravimetric water content	55 %
Fraction organic carbon	5.22 %
Porewater salinity	0.25 ppt
Particle size analysis	
Medium silt and smaller	33.7 %
Coarse silt	19.8 %
Fine sand	19.6 %
Medium sand	20.8 %
Coarse sand	6.20 %

Table 2. Reducing equivalents added to PCE microcosms and time required to achieve complete dechlorination of PCE to ethene for the initial three dechlorination cycles. Reducing equivalents were added in the form of acetate and hydrogen. Times required for complete dechlorination are averages of duplicates.

Microcosm Name	Reducing Equivalents Added (meq total)[a]	ED to PCE Ratio[b]	Days Required for Complete Dechlorination		
			1st Dechlorination Cycle	2nd Dechlorination Cycle	3rd Dechlorination Cycle
No H_2	0.00	0.00	82	24	20
Minimal H_2	0.1	1.4	80	19	26
Acetate + H_2	1.3	16.6	76	15	10
Excess H_2	2.5	31.3	56	12	6
Bioaugmented[c]	0.1	1.4	33	16	21

[a] Reducing equivalents were calculated based on 2 equivalents per 1 mole H_2; 4 equivalents per 1 mole acetate (*66*). "Minimal H_2" and "Acetate + H_2" microcosms had 3.5 % H_2 in microcosm headspace and "Excess H_2" microcosms had 80% H_2 in microcosm headspace. [b] Electron donor (ED) to PCE ratio was calculated based on 8 reducing equivalents required for PCE to be reductively dechlorinated to ethene, all chloroethene mass being bioavailable, and no loss of reducing equivalents to competing biotic and abiotic processes. [c] Bioaugmented microcosms were supplied with a 5% inoculum (volume culture/aqueous volume microcosm) of a PCE to ethene mixed culture, sparged with N_2 prior to addition.

Table 3. Microbial characterization and quantification of a microcosm supplied wet sediment, simulated groundwater, PCE, acetate, and hydrogen. Quantification of *Dehalococcoides* and RDase numbers are average of triplicates ± one standard deviation.

Target Microorganism or Gene	**Dechlorination Activity**	Result	Quantification (gene copies·mL^{-1})
Dehalobacter restrictus	PCE → DCE	+	
Desulfuromonas spp.	PCE → DCE	+	
Desulfomonile spp.	PCE → DCE	+	*Not Performed*
Geobacter sp. strain SZ	PCE → DCE	N/D [a]	
Dehalococcoides spp.	PCE → ETH DCEs → ETH	+	$5.19 \times 10^8 \pm 2.25 \times 10^7$
tceA enzyme (*Dehalococcoides* sp. strain 195 or strain FL2)	TCE → VC	+	$8.29 \times 10^7 \pm 2.91 \times 10^6$
vcrA enzyme (*Dehalococcoides* sp. strain VS or strain GT)	DCEs → ETH	+	$2.13 \times 10^8 \pm 2.05 \times 10^7$
bvcA enzyme (*Dehalococcoides* sp. strain BAV 1)	VC → ETH	+	$2.78 \times 10^7 \pm 1.09 \times 10^5$

[a] N/D = not detected with PCR (nested or direct).

Table 4. Summary of tracer experiments conducted on sediment column using bromide as the nonreactive tracer. The Peclet number is an indicator of dispersion within the sediment; beta represents the dimensionless fraction of mobile pore volumes within the column; omega represents a dimensionless rate constant; and alpha is a calculated mass transfer coefficient (hr^{-1}).

Property	Tracer 1	Tracer 2
Period of tracer effluent collection (pore volumes)	5 to 8	96 to 101
Peclet number[a]	37.29	2.28
Beta[a]	0.793	0.358
Omega[a]	0.547	0.409
Alpha (hr^{-1})	0.0197	0.0.0147

[a] Fitted parameters were obtained with the CFITM3 break through curve fitting program under physical nonequilibrium constraints.

Table 5. Summary of experimental conditions for sand column experiments.

Parameter	Sand Column A	Sand Column B
Void Volume[a] (mL)	62.72	61.82
Porosity[a] (cm^3 void·(cm^3 total)$^{-1}$)	0.413	0.407
Connected In Series to Sediment Column (sediment pore volumes)	67.02 to 83.21	145.96 to 180.00
Experimental Flow Rate(s) (mL·hr^{-1})	5.4642	1.2879; 2.5758; 5.4642
Seepage Velocity (cm·day^{-1}) (Darcy Velocity (cm·day^{-1}))	62.67 (25.88)	14.99; 29.98; 63.59 (6.10); (12.20); (25.88)
Peclet Number[b] (dimensionless)	80.48	N/A
Alterations to Influent	None	Addition of Lactate Addition of cis-DCE Decrease of Flow Rate
Influent Chloroethene Concentration (μM total chloroethenes)	16.19 ± 11.06[c]	0 to 3.44 pv: 200 ± 42[c] 3.44 pv to end : 34 ± 3.6[c]

[a] Estimated from mass difference between dry and wet packed columns. [b] Obtained with the CFITM3 break through curve fitting program under equilibrium constraints. [C] Average ± one standard deviation.

E. Supplemental Keywords: bioremediation, contaminant fate and transport, sediment characterization, microbial processes

F. List of Students Supported: David W. Himmelheber, Ph.D. candidate, degree expected in 2007

G. List of Publications and Presentations:

David W. Himmelheber, Kurt D. Pennell, and Joseph B. Hughes. "Natural Attenuation Processes During In Situ Capping". In Preparation for *Environmental Science & Technology*, with submission expected by 02/07.

David W. Himmelheber and Joseph B. Hughes. "Impact of Sediment Capping on the Reductive Natural Attenuation of Groundwater Seeps" Platform presentation to be given at the 4th International Conference on Remediation of Contaminated Sediments, Savannah, GA, January 2007.

David W. Himmelheber and Joseph B. Hughes. "Complete PCE Dechlorination Under Conditions Associated with *In Situ* Capping." Poster presented at the 5th International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 2006.

David W. Himmelheber and Joseph B. Hughes. "Complete Tetrachloroethene Dechlorination in Anacostia River Sediment." Poster presented at the SETAC North American 26th Annual Meeting, Baltimore, MD, November 2005.

David W. Himmelheber and Joseph B. Hughes. "Complete Tetrachloroethene Dechlorination in Anacostia River Sediment." Poster presented at the 105th ASM General Meeting, Atlanta, GA, June 2005.

David W. Himmelheber and Joseph B. Hughes, "Gas Production and PCE Dechlorination in Anacostia River Sediment." Poster presented at the EPA/ORD-HSRC Superfund Research on Risk Characterization and Monitoring Workshop, Las Vegas, NV, November 2004.

Appendices: none